The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Postprandial Cysteine/Cystine Redox Potential in Human Plasma Varies with Meal Content of Sulfur Amino Acids[1–3]

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Abstract

Few data are available on plasma redox responses to sulfur amino acid (SAA) loads. In this study, we had 2 aims: to determine whether the SAA content of a meal affected postprandial plasma cysteine (Cys), cystine (CySS), or redox potential (E_hCySS) in humans and whether SAA intake level (adequate or inadequate) in the days preceding the meal challenge affected these postprandial levels. Eight healthy individuals aged 18–36 y were equilibrated for 3 d to adequate SAA, fed chemically defined meals without SAA for 5 d (inadequate SAA) and then fed isoenergetic, isonitrogenous meals with adequate SAA for 5 d. On the first and last days with the chemically defined meals, a morning meal containing 60% of the daily food intake was given, and plasma Cys, CySS, and E_hCySS were determined over an 8-h postprandial time course. Following equilibration to adequate intake, provision of the meal with SAA resulted in increased plasma Cys and CySS concentrations and more reduced plasma E_hCySS compared with the postprandial values following the same meal without SAA. Equilibration to inadequate SAA intake for the days preceding the meal challenge did not affect this response. The magnitude of the difference in postprandial plasma E_hCySS (10 mV) due to meal content of SAA was comparable to those which alter physiologic signaling and/or are associated with disease risk. Consequently, the SAA content of meals could affect physiologic signaling and associated disease mechanisms in the postprandial period by changes in Cys, CySS, or E_hCySS. J. Nutr. 140: 760–765, 2010.

Introduction

Cysteinyl residues of proteins in cell membranes undergo reversible oxidation-reduction in response to the redox potential (E_hCySS[3] in mV)[9] of the extracellular Cys/cystine (CySS) pool, the predominant low molecular weight thiol/disulfide couple in human plasma. A relatively oxidized E_hCySS increases proinflammatory cytokine production in monocytes (1), expression of cell adhesion molecules in endothelial cells (2,3), profibrotic signaling in lung fibroblasts (4), and apoptosis in retinal pigment epithelial cells (5). In contrast, a relatively reduced E_hCySS increases cell cycle gene expression in monocytes (6), transforming growth factor-α signaling in Caco-2 cells, and cell proliferation in monocytes, endothelial cells, retinal pigment epithelial cells, and colon carcinoma Caco-2 cells (7,8).

Plasma E_hCySS is more oxidized (positive) in association with aging (9) and disease risk factors, including cigarette smoking (10), chronic alcohol abuse (11), and anticancer therapy (12). Increased plasma CySS concentration and/or oxidized plasma E_hCySS have been associated with human disease risk, e.g. persistent atrial fibrillation (13), peripheral vascular disease (14), and age-related macular degeneration (15). In vivo studies in humans also show that oxidized thiol/disulfide redox potential is associated with increased carotid intima media thickness (14), decreased flow-mediated dilation (16), reversible myocardial perfusion defects (17), and persistent atrial fibrillation (13). Thus, dietary factors affecting extracellular thiol/disulfide redox potential in human plasma could be important in disease risk and development.

Relatively little is known about the dietary factors affecting E_hCySS in vivo. In a previous cysteine (Cys) challenge study, we showed that following an oral load of 3 g Cys without food, Cys and CySS increased to a maximum at 1 h (18). The calculation of E_hCySS from these data shows that the redox potential becomes more reduced by 40 mV at 1 h after Cys administration. A study

[1] Supported by NIH grants ES012929 and ES011195 (D.P.J.), DK55850, and K24 RR023356 (T.R.Z.) and Emory General Clinical Research Center grant M01 RR00039/UL1 RR025008.

[2] Author disclosures: Y. Park, T. R. Ziegler, N. Gletsu-Miller, Y. Liang, T. Yu, C. J. Accardi, and D. P. Jones, no conflicts of interest.

[3] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.

[9] Abbreviations used: AUC, area under the curve; CDO, cysteine dioxygenase; CL, metabolic clearance; CySS, cystine; E_hCySS, redox potential of cysteine/cystine couple; GCRC, General Clinical Research Center; k_{elim}, elimination rate constant; SAA, sulfur amino acid; $t_{1/2}$, elimination half-life; V_D, apparent volume of distribution.

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Manuscript received September 29, 2009. Initial review completed December 4, 2009. Revision accepted January 15, 2010.
First published online February 17, 2010; doi:10.3945/jn.109.116764.

of diurnal variation of glutathione and Cys in human plasma showed that plasma E_hCySS varied in an apparent meal-related pattern, becoming transiently reduced 2–3 h after each meal and maximally reduced at 2030, which was 3 h after the largest meal (19). These data suggest that plasma Cys and E_hCySS vary in the postprandial period.

The present study was designed to determine whether the presence of sulfur amino acids (SAA) in a meal affects postprandial plasma Cys, CySS, or E_hCySS in healthy adults. The study used a semisynthetic, chemically defined diet based on the studies of Young et al. (20,21), which allows specific changes in Met and Cys content. Changes in plasma Cys, CySS, and E_hCySS were measured as a function of time for an 8-h period following a meal containing adequate (U.S. mean consumption) or no SAA content. Because the abundance of Cys dioxygenase (CDO), a key catabolic enzyme for Cys, is regulated by the Cys intake (22), the study was designed to also determine whether postprandial responses to meal challenge were affected by prior equilibration to insufficient SAA intake.

Materials and Methods

Materials. Sodium heparin, bathophenanthroline disulfonate sodium salt, sodium iodoacetate, dansyl chloride, L-serine, Cys, CySS, and sodium acetate trihydrate were from Sigma Chemical. γ-Glutamylglutamate was from MP Biomedicals. Boric acid, sodium tetraborate, potassium tetraborate, perchloric acid, and acetic acid were reagent grade and purchased locally. Methanol, acetone, and chloroform were HPLC grade.

Human participants. This study was reviewed and approved by the Emory Investigational Review Board and was performed in accordance with the ethical guidelines outlined in the U.S. Health and Human Services Policy for Protection of Human Research Subjects. A total of 8 volunteers, self-described as healthy, were recruited beginning January 1, 2005, by posting fliers in public locations in the Atlanta/Emory University community. Following informed consent, all participants were screened in the outpatient unit of the Emory University Hospital General Clinical Research Center (GCRC), where a medical history and physical examination, body height and weight, fasting standard blood chemistry and hematology tests, and a urinalysis were performed (a serum pregnancy test was also performed in females). Indirect calorimetry was used to determine resting energy expenditure, and energy content of meals was based upon the daily energy requirement calculated as 1.3 resting energy expenditure. Eligibility was established by the absence of evidence of acute or chronic illness, no current smoking history, and a BMI < 30.

Participants were scheduled to begin the study within 1 mo of screening. Participants taking antioxidants, nutrient supplements (with the exception of once-daily multivitamin-mineral supplements), or acetaminophen were asked to discontinue these 2 wk prior to the onset of the studies because of possible effects on E_hCySS. Four study periods (see **Supplemental Fig. 1** for details) within a 13-d inpatient stay in the Emory Hospital GCRC were used to determine whether meal content of SAA had an effect on postprandial plasma Cys, CySS, and E_hCySS and whether adequate or inadequate SAA intake for the preceding 3 d affected these plasma measures. The first study period began after 3 d of equilibration with regular food containing the Recommended Dietary Allowance for SAA, at which time the participant was given a meal without SAA and studied for 8 h in the postprandial period. This study period was designated the adequate/−SAA period (Supplemental Fig. 1). The participant received food without SAA for the next 4 d and was again studied in the postprandial period after receiving a meal without SAA. This second period is designated the inadequate/−SAA period. On the following day when the participant was still equilibrated to inadequate SAA, the participant was given an isonitrogenous, isoenergetic meal containing SAA; this period is designated the inadequate/+SAA period. After 4 d with adequate intake, the participant was again studied with a meal containing SAA; this period is designated the adequate/+SAA period (Supplemental Fig. 1).

For each study period, a plasma sample was collected at 0830 h just before provision of a meal consisting of 60% of the estimated total daily energy requirement. Postprandial samples were collected at 0930, 1030, 1130, 1230, 1430, and 1630 h. The chemically defined diets contained an L-amino acid mixture expressed per kg body weight to provide 1.0 $g \cdot kg^{-1} \cdot d^{-1}$ protein equivalents (see below). Study meals were prepared in the GCRC metabolic kitchen and consumed over 20 min; intake was monitored by the GCRC Bionutrition Unit staff.

Meal composition. The protein equivalents of diets were supplied in the form of specific L-amino acid mixtures (Ajinomoto USA), designed to provide 1.0 $g \cdot kg^{-1} \cdot d^{-1}$, as previously outlined in detail (20,21). The standard mixture was patterned after hen's egg protein and provided all 9 indispensable (essential) amino acids, including Met, in amounts sufficient for the mean requirements of healthy young adults (20,21), but which were higher than the requirements proposed by the WHO (21,23). The standard amino acid mixture also contained 9 dispensable (nonessential) amino acids, including Cys and glutamate, and lacked glutamine- and taurine. To compensate for the difference in Met + Cys, the amount of all nonessential amino acids was proportionally changed to maintain a constant dietary nitrogen content while also maintaining them as isoenergetic. The proportion by weight of Met:Cys was constant (2:1). To improve palatability, a powdered flavoring agent was added to the liquid amino acid mixture (provided as a sherbet-based drink) (20,21). The Cys was added immediately prior to consumption to minimize Cys oxidation to CySS. The dietary energy was mainly derived from lipid and carbohydrate sources provided in the form of protein-free wheat starch and butter/safflower oil cookies and a sherbet-based drink, as outlined (20,21).

Sampling and redox analyses

A heparin-lock catheter was placed in a forearm vein for blood sampling (19) and plasma samples were collected at 0830 (just prior to the meal), 0930, 1030, 1130, 1230, 1440, and 1630, with immediate transfer into a preservation solution containing in internal standard. Samples were frozen at −80° and analyzed by HPLC following derivatization with dansyl chloride (24) within 2 mo of collection. Stability tests showed that samples were stable for this duration. Cys and CySS were detected by fluorescence and quantified by integration relative to the internal standard, with validation relative to external standards (24). E_hCySS was calculated using the Nernst equation, $E_h = E_o + RT/nF \ln[CySS]/([Cys]^2)$, where E_o is the standard potential (−250 mV for CySS/Cys at pH 7.4), R is the gas constant, T is the absolute temperature, n is 2 for the number of electrons transferred, and F is Faraday's constant (25). Protein cysteinylation correlated with plasma CySS and was not measured in the current study (25).

Kinetic constants. The rate constants for elimination (k_{Elim}) and the apparent volumes of distribution (V_D) were determined from the slope and the *y*-intercept, respectively, of the semilogarithmic plot of plasma concentrations of Cys and CySS as a function of time. The elimination half-life ($t_{1/2}$) was related to the k_{Elim} by the equation $t_{1/2} = 0.693/k_{Elim}$. Metabolic clearance (CL) was calculated as the product $k_{Elim} \cdot V_D$.

Statistics. Descriptive statistics were performed using Minitab software (version 15; Minitab). To compare the effects of time and SAA intake on responses and their interaction, a repeated-measures ANOVA was performed using R software (K. Hornik, The R FAQ, 2009, ISBN 3-900051-08-9, http://CRAN.R-project.org/doc/FAQ/R-FAQ.html) with a mixed effects model. Paired t tests to compare group responses for specific postchallenge time points were performed using Minitab. Data are expressed as mean ± SEM and were considered significant at $P < 0.05$.

Results

Study participant characteristics

Eight participants aged 18–36 y were studied (**Table 1**), including 5 males and 3 females with BMI ranging from 20 to

TABLE 1 Characteristics of the humans[1]

	Males	Females	Total
Participants, *n (%)*	5 (62)	3 (38)	8
Age, *y*	25 ± 6	25 ± 8	25 ± 6
Weight, *kg*	66 ± 9	65 ± 11	66 ± 9
BMI	21 ± 1	24 ± 2	23 ± 2

[1] Data are means ± SD.

26 $kg \cdot m^{-2}$. The participants reported no acute or chronic illness and none were taking regular prescription medications.

Postprandial effects with equilibration to adequate SAA intake

Effect of meal Cys content on plasma Cys. At baseline, the plasma Cys concentration was 9.9 ± 1.5 $\mu mol \cdot L^{-1}$ following the 3-d equilibration (**Fig. 1***A*). SAA intake (adequate/+SAA vs. adequate/−SAA) affected Cys levels ($P < 0.0001$) by repeated-measures ANOVA. During the adequate/+SAA period, plasma Cys was maximal at 1 h (19 ± 3 $\mu mol \cdot L^{-1}$) (Fig. 1*A*). Plasma Cys concentrations were significantly higher than the corresponding time points in the adequate/−SAA period for 1-, 2-, 3-, and 4-h time points, respectively (Fig. 1*A*).

Kinetic parameters during the adequate/+SAA period were estimated from semilogarithmic plots of concentration vs. time and showed that after achieving the maximal value, plasma Cys decreased with an apparent first-order loss. The $t_{1/2}$ was 3 ± 0.2 h and V_D was 190 ± 30 L (**Table 2**). Assuming nearly complete absorption into a blood volume of ~6 L, this high V_D indicated a rapid removal of Cys from the blood volume. Using the k_{Elim} determined from the $t_{1/2}$, the CL was 44 ± 8 $L \cdot h^{-1}$ (Table 2).

Effect of meal Cys content on plasma CySS. At baseline, the plasma CySS concentration following the 3-d equilibration was 78 ± 5 $\mu mol \cdot L^{-1}$ (Fig. 1*B*). SAA intake (adequate/+SAA vs. adequate/−SAA) affected CySS levels ($P < 0.0001$). During the adequate/+SAA period, CySS concentration increased achieving a maximal value of 94 ± 8 $\mu mol \cdot L^{-1}$ at 3 h (Fig. 1*B*). The area under the curve (AUC) for plasma CySS was 67 ± 8 $\mu mol \cdot L^{-1} \cdot h$ (Table 2). When expressed in Cys equivalents, the value (134 ± 16 $\mu mol \cdot L^{-1} \cdot h$) was greater than that for plasma Cys (47 ± 12 $\mu mol \cdot L^{-1} \cdot h$) ($P < 0.05$; Table 2). This shows that following consumption of a meal with Cys, a larger amount of the total (Cys + CySS) pool is present in plasma as CySS than as Cys. Because Cys was added to the meal immediately prior to consumption and was confirmed by HPLC to be in the reduced Cys form rather than CySS, these data show that extensive oxidation of Cys occurs upon absorption into systemic circulation. The k_{Elim} and CL values were lower for CySS than for Cys ($P < 0.05$). Thus, in association with Cys consumption, a relatively large fraction of the ingested Cys appears in circulation as CySS, and this CySS is cleared more slowly than Cys.

Effect of meal Cys content on plasma E_hCySS. At baseline, the plasma E_hCySS was −74 ± 4 mV (Fig. 1*C*). There was a significant effect of SAA intake (adequate/+SAA vs. adequate/−SAA) ($P < 0.0001$), with the postchallenge data from the adequate/+SAA period showing more reduced values than with zero SAA (adequate/−SAA). Plasma E_hCySS became reduced (more negative) in the adequate/+SAA period, achieving a maximally reduced value of −85 ± 4 mV after 2 h (Fig. 1*C*). This value was 10 mV more reduced than the fasting morning value and 13 mV more reduced than the 2-h value in the adequate/−SAA period (Fig. 1*C*). Thus, the results show that plasma E_hCySS becomes more reduced as a consequence of consumption of food containing SAA at an amount found in typical American diets.

Postprandial effects with equilibration to inadequate SAA intake

Effect of meal SAA content on plasma Cys. At baseline, the plasma Cys concentration was 8.0 ± 1.0 $\mu mol \cdot L^{-1}$ following

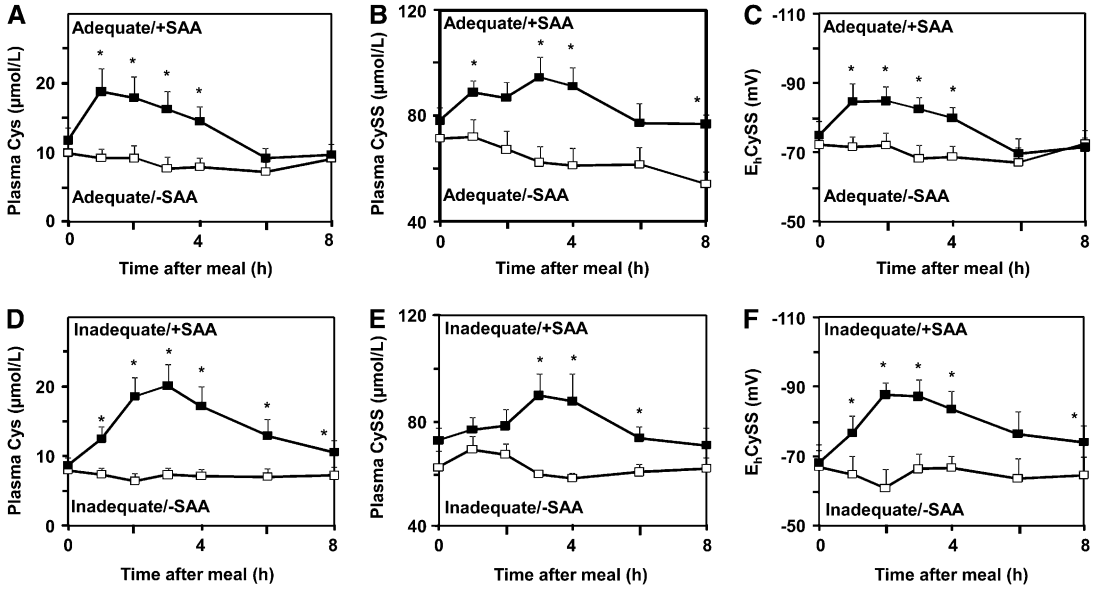

FIGURE 1 Effect of meal SAA content on postprandial plasma Cys (*A,D*) and CySS (*B,E*) concentrations and EhCySS (*C,F*) in humans. Panels *A–C* show values obtained following a meal containing SAA (+SAA) and an equivalent meal without SAA (−SAA) after equilibration with an adequate SAA diet. Panels *D–F* are comparable data obtained after equilibration with an inadequate SAA diet. Values are means + SEM, $n = 8$. *Different from −SAA at that time, $P < 0.05$ (paired *t* test). For each panel, the effect of SAA was significant, $P < 0.0001$ (repeated-measures ANOVA with a mixed effect model).

TABLE 2 Kinetic constants for plasma Cys and CySS following oral intake by humans of a meal containing SAA (+SAA) and an equivalent meal without SAA (−SAA) after equilibration with an adequate or inadequate SAA diet

	Study period	
	Adequate/+SAA	Inadequate/+SAA
Plasma Cys kinetics		
AUC, $\mu mol \cdot L^{-1} \cdot h$	47 ± 12	51 ± 12
Increase, $\mu mol \cdot L^{-1}$	14 ± 3	16 ± 3
V_D, L	190 ± 30	200 ± 40
$t_{1/2}$, h	3.0 ± 0.2	4.0 ± 1.0
k_{Elim}, h^{-1}	0.23 ± 0.01	0.20 ± 0.03
CL, $L \cdot h^{-1}$	44 ± 8	40 ± 12
Plasma CySS kinetics		
AUC, $\mu mol \cdot L^{-1} \cdot h$	67 ± 8	75 ± 12
Mean plasma increase, $\mu mol \cdot L^{-1}$	18 ± 3	20 ± 2
V_D, L	58 ± 1	55 ± 4
$t_{1/2}$, h	8.1 ± 0.7	4.1 ± 0.6*
k_{Elim}, h^{-1}	0.09 ± 0.01	0.15 ± 0.02*
CL, $L \cdot h^{-1}$	5.4 ± 0.8	8 ± 1

[1] Data are means ± SE, $n = 8$. *Different from Adequate/+SAA, $P < 0.05$ (paired t test). Other comparisons between Cys and CySS parameters are described in the text.

equilibration to inadequate SAA intake (Fig. 1*D*). Under this condition, SAA intake (inadequate/+SAA vs. inadequate/−SAA) affected Cys levels by repeated-measures ANOVA ($P < 0.0001$). During the inadequate/+SAA period, plasma Cys increased following intake of the SAA-containing meal and was maximal at 3 h (20 ± 3 $\mu mol \cdot L^{-1}$) (Fig. 1*D*). Plasma Cys concentrations were significantly higher than the corresponding time points in the inadequate/−SAA period for 2-, 3-, 4-, and 5-h time points, respectively (Fig. 1*D*).

The AUC, k_{Elim}, and CL for Cys during the inadequate/+SAA period did not significantly differ from values for the adequate/+SAA period by paired t tests (Table 2), suggesting that any potential changes in absorption and clearance or disposition due to equilibration to an inadequate SAA intake had little effect on plasma Cys responses to consumption of a meal with mean Cys content.

Effect of SAA content in meal on plasma CySS. At baseline, the plasma CySS concentration was 69 ± 6 $\mu mol \cdot L^{-1}$ following the period of SAA depletion (Fig. 1*E*). SAA intake (inadequate/+SAA vs. inadequate/−SAA) affected CySS levels ($P < 0.0001$). CySS concentration increased during the inadequate/+SAA period, achieving a maximal value of 89 ± 6 $\mu mol \cdot L^{-1}$ at 3 h (Fig. 1*E*).

The AUC for CySS was 75 ± 12 $\mu mol \cdot L^{-1} \cdot h$ during the inadequate/+SAA period; this did not differ from that for the adequate/+SAA period (67 ± 8 $\mu mol \cdot L^{-1} \cdot h$) (Table 2). The estimated $t_{1/2}$ was 4.1 ± 0.6 h during the inadequate/+SAA period (Table 2), less than the estimate for the adequate/+SAA period (8.1 ± 0.7 h) (Table 2) ($P < 0.05$). The k_{Elim} was greater for the inadequate/+SAA period (0.15 ± 0.02 h^{-1}) compared with the adequate/+SAA period (0.09 ± 0.01 h^{-1}) (Table 2) ($P < 0.05$).

Effect of meal Cys content on plasma E_hCySS after inadequate intake of SAA. At baseline, the plasma E_hCySS was −67 ± 5 mV (Fig. 1*F*). There was a significant effect of SAA intake (insufficient/+SAA vs. insufficient/−SAA) ($P < 0.0001$), with the postchallenge inadequate/+SAA group showing more reduced values than the inadequate/−SAA group. Plasma E_hCySS became more reduced in the inadequate/+SAA period, achieving a maximally reduced value of −88 ± 4 mV after 2 h (Fig. 1*F*). This value was 20 mV more reduced than the fasting morning value and 27 mV more reduced than the 2-h samples in the inadequate/−SAA period (Fig. 1*F*).

Discussion

Glutathione is the most abundant low molecular weight thiol in cells, but the amino acid Cys and its disulfide, CySS, constitute the major low molecular weight thiol/disulfide system in human plasma (26). A number of in vitro and in vivo studies suggest that oxidation of the plasma Cys/CySS pool could be important in human health. For instance, human cells in culture regulate extracellular E_hCySS in the culture medium to the value found in plasma of young healthy adults (7). Controlled variation of extracellular E_hCySS in culture medium alters cell proliferation (2,4,7,8,27), sensitivity of cells to oxidant-induced apoptosis (5), adhesion of monocytes and neutrophils to endothelial cells (1,2), and profibrotic signaling through transforming growth factor-β (4). Cell surface thiols participate in these redox sensitivities, because pretreatment with cell impermeable alkylating reagents blocks redox effects (1,2,8). A more oxidized plasma E_hCySS has been associated with a number of disease risk factors (28).

The present study addressed whether SAA in food affected plasma E_hCySS. In individuals with a previous history of adequate SAA intake, the plasma E_hCySS without SAA was 10 mV more oxidized than the same meal with SAA. The magnitude of meal-related changes in E_hCySS in the present study are similar to differences associated with aging (9,15), smoking (10), alcohol consumption (11), and persistent atrial fibrillation (13). Thus, the data suggest that in free-living individuals eating diets with SAA at levels similar to the mean American intake, the magnitude of change in E_hCySS during the postprandial period is sufficient to contribute to disease risk.

We focused on E_hCySS in the present study, because previous in vitro mechanistic research showed relevant redox effects on cell signaling. However, some of the clinical studies provide stronger associations of disease risk with CySS concentration than with E_hCySS (14,29,30). This creates a complexity in interpretation, because earlier studies showed that diurnal changes in E_hCySS were largely associated with variations in Cys concentration (19) whereas age-dependent oxidation of E_hCySS was largely associated with increased CySS (9). To address critical questions concerning optimal SAA intake and disease, additional studies are needed in older, at-risk individuals.

Research in rodents shows that high Cys intake activates Cys catabolism by CDO (31–33). CDO levels in rats are responsive to changes in SAA intake, reaching new steady-state levels within 24 h (22). We designed the study in humans to test for differences in metabolism between equilibration to adequate and inadequate SAA intake. There were no significant differences detected (compare Fig. 1*A* and *D*, Fig. 1*B* and *E*, Fig. 1*C* and *F*). Thus, the data in these individuals suggest that plasma Cys and E_hCySS are most responsive to the current meal SAA content and not the several days of previous SAA intake before the meal challenges. More direct experiments will be needed to test the role of CDO changes in adaptation of Cys metabolism to SAA intake level in humans.

The kinetic data provided by the current study suggest that tracer studies are needed to evaluate the plasma Cys flux to

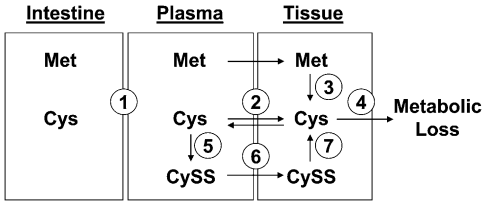

FIGURE 2 Minimal kinetic model for plasma E_hCySS in humans. The present data show that a large fraction of dietary Cys appears as CySS in plasma. This reveals a need to incorporate steps 5–7 in models for plasma Cys turnover. Thus, minimal components for absorption and turnover of Cys include: *1*) transepithelial transport of Cys from the small intestine into the plasma; *2*) reversible transport of Cys from plasma into tissues; *3*) biosynthesis of Cys from Met within tissues; *4*) catabolism and removal of Cys by CDO and other metabolic pathways within tissues; *5*) oxidation of Cys to CySS; *6*) uptake of CySS from plasma by tissues; and *7*) reduction of CySS to Cys within tissues. The changes in plasma E_hCySS and the delay in clearance of plasma CySS following meals with high SAA content indicate that additional stable isotopic tracer studies are needed to evaluate the rates of steps 5–7 separately from steps 2–4.

plasma CySS as a separate component of the metabolic pathway for Cys turnover (**Fig. 2**). The calculated V_D for Cys (190–200 L) shows that Cys was rapidly removed from plasma, which could be due to transport into tissues and/or oxidation to CySS or other products. Both the current study and a previous study (18) indicate that a fraction of the Cys is converted to CySS over the same time course as absorption. The AUC data for CySS show that a substantial fraction of the absorbed Cys is oxidized to CySS. Because the AUC for CySS (expressed in Cys equivalents) was greater than the administered Cys load, the data indicate that Met conversion to Cys also contributes to the overall plasma CySS flux (Fig. 2). An estimate of CySS turnover obtained from the mean AUC for the measured 8-h time course was 18 μmol·kg^{-1}·h^{-1}, which is one-half of the Cys flux (38–80 μmol·kg^{-1}·h^{-1}), measured using stable isotopic tracer methods (20,21,34). This, combined with the plasma concentration of CySS being considerably greater than that of Cys, indicates that kinetic models may be improved by the inclusion of oxidation of Cys to CySS and tissue CySS uptake and reduction (i.e. steps 5–7, Fig. 2).

One of the limitations of the current study is that the clearance of Cys by oxidation and catabolism is confounded by ongoing synthesis of Cys from Met, and tracer studies will be needed to discriminate these processes. Another limitation is that overall effects of negative nitrogen balance induced by the absence of both Cys and Met in the diet devoid of SAA are unclear.

In summary, the postprandial plasma E_hCySS was more reduced with a semisynthetic, chemically defined diet containing SAA than an equivalent isoenergetic, isonitrogenous diet without SAA. The magnitude of plasma redox changes during the postprandial period due to SAA content of the food was similar to previously reported variations in E_hCySS associated with aging, oxidative stress, and disease. Thus, the data suggest that meal-related variations in E_hCySS may be of a sufficient magnitude to be considered in redox mechanisms of disease.

Acknowledgments

Y.P., D.J., and T.Z. participated in study design, analytic supervision, data interpretation, and manuscript preparation. Y.P. and T.Y. performed statistical analyses. N.G-M. participated in design detail, participant recruitment, and coordination of the clinical aspects of this study in collaboration with T.Z. Y.L. participated in analytical design and amino thiol analysis. C.A. participated in data interpretation and project supervision. All authors read and approved of the final version of this manuscript.

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